The network of immunosuppressive pathways in glioblastoma

Mangani, Davide ; Weller, Michael ; Roth, Patrick

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The network of immunosuppressive pathways in glioblastoma

Davide Mangani[1], Michael Weller[1,2], Patrick Roth[1,2]

[1]Laboratory of Molecular Neuro-Oncology, Department of Neurology and Brain Tumor Center, University Hospital and University of Zurich, Zurich, Switzerland; [2]Center for Neuroscience, University of Zurich, Zurich, Switzerland

Correspondence: Dr. Patrick Roth, Department of Neurology, University Hospital, Frauenklinikstrasse 26, 8091 Zurich, Switzerland, Tel.: +41 (0)44 255 5511, Fax: +41 (0)44 255 4380, E-mail: patrick.roth@usz.ch

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Abbreviations: antigen-presenting cells (APC); blood brain barrier (BBB); colony stimulating factor-1 receptor (CSF-1R); cytotoxic T lymphocyte antigen 4 (CTLA-4); deep cervical lymph node (dcLN); dendritic cells (DC); forkhead box P3 (FoxP3); glioblastoma (GBM); growth and differentiation factor-15 (GDF-15); hypoxia inducible factor (HIF)-1α; interferon (IFN); indoleamine-2,3-dioxygenase (IDO); induced Treg (iTreg); interleukin-2 receptor alpha chain (CD25); lectin-like transcript-1 (LLT-1); major histocompatibility complex (MHC); matrix metalloproteinase (MMP); myeloid-derived suppressor cells (MDSC); natural killer (NK); natural Treg (nTreg); nitric oxide (NO); programmed cell death 1 (PD-1); PD ligand-1 (PD-L1); regeneration and tolerance factor (RTF); regulatory T cells (Treg); transforming growth factor-β (TGF-β); tryptophan-2,3-dioxygenase (TDO); tumor-associated macrophages (TAM); vascular endothelial growth factor (VEGF)

Abstract

Glioblastoma remains a fatal tumor despite increased knowledge regarding the complex signalling pathways that drive this devastating disease. Recently, immunotherapeutic approaches have shown remarkable and durable responses in various cancers including metastatic melanoma and advanced non-small cell lung cancer. So far, it remains unclear whether these immunotherapeutics may also work against glioblastoma and other tumors residing in the central nervous system. It is well known that patients with glioblastoma suffer from profound local immunosuppression that represents the major hurdle to overcome in the context of immunotherapy. Several studies have demonstrated that this immunosuppressive phenotype is orchestrated by glioma-derived membrane-bound and soluble factors as well as the particular microenvironment within the brain. Here, we discuss the molecular and cellular pathways involved in glioblastoma-mediated inhibition of the immune system and highlight possible treatment approaches aiming at reinvigorating anti-tumor immune responses.

1. Introduction

Glioblastoma is the most common and deadly primary malignant brain tumor in adults [1]. Despite maximal safe surgery, radio- and chemotherapy, the prognosis remains dismal with a median survival around 16 months within clinical trial populations [2]. Great promises held by targeted treatments directed against vascular endothelial growth factor (VEGF) or specific integrins were not fulfilled since these drugs failed to prolong overall survival in randomized clinical trials [3-5]. Hence, new therapeutic modalities are urgently needed. Immunotherapy has been regarded a promising treatment option for decades, however, without making significant progress. However, the therapeutic success achieved by “immune checkpoint inhibitors” in several tumor entities such as metastatic melanoma and non-small cell lung cancer with so far unseen response rates, paved the road for a renewed interest in exploring immunotherapeutic strategies also against glioblastoma [6-8].

Most therapeutic strategies employing the immune system are based on the consideration that T cells can recognize and respond against genetic and cellular alterations which occur during cancer development and progression [9]. Preclinical data from experiments using various methylcholanthrene-induced tumors demonstrated that knock-out mice lacking either components of the interferon (IFN)-γ pathway or the perforin gene are more susceptible to tumor formation [10, 11]. Subsequent studies proved the existence of tumor-specific T cells directed against mutated or overexpressed proteins confirming the presence of anti-tumor immune responses [12]. However, selective pressure on tumor cells by the immune system may lead to the emergence of immune-edited clones that escape recognition and ultimately grow undisturbed [13].

GBM is peculiar for its ability to escape from immune surveillance and two major challenges represent a major obstacle for the successful administration of immunotherapies: the tumor location in the brain which comprises an immunoprivileged microenvironment as well as the presence of several glioma-derived mechanisms of active immunosuppression.

In this review article, we illustrate the pathways that are mainly involved in mediating glioblastoma-associated immunosuppression and therapeutic strategies which aim at reinvigorating immune responses against the tumor.

2. Blood brain barrier and immunological routes to the brain

A first hurdle to overcome in the context of immunotherapies for brain tumors is represented by the tumor location within the CNS. The presence of the blood brain barrier (BBB) and the absence of classical lymphatic vessels are two major issues that may hamper the successful administration of any immunotherapy. The BBB is a cellular barrier formed by specialized brain endothelial cells, pericytes and astrocytes, which regulates the ionic composition of the brain thereby maintaining appropriate neuronal function by blocking the entrance of unwanted and possibly neurotoxic molecules. The latter property relies on the presence of several ATP-binding cassette (ABC) transporters, that actively export molecules and drugs out of the brain [14]. The BBB also regulates the entry of immune cells to the CNS. Under physiological conditions, only few immune cells are present in the brain parenchyma, whereas various pathological conditions result in disruption of the BBB which then becomes more permeable to immune cell entry [15-17]. Substantial work has shown that antigens released within the CNS are drained towards peripheral lymphoid tissues

and can be presented by antigen-presenting cells (APC) to naïve T cells, which can subsequently be activated and upregulate the expression of α4 and β1 integrins [18, 19]. Only when expressing these integrins, lymphocytes can interact with vascular cell adhesion molecule (VCAM)-1 expressed on the cerebral endothelium and pass across the BBB. Immune cell infiltration has been found in glioblastoma, however, to a variable extent [20-22].

Despite being partially disrupted during tumor progression, intact zones of the BBB may protect tumor cells invading the normal brain parenchyma from drug delivery, representing a possible explanation for the re-iterated failure of various systemic therapies in GBM and other brain tumors [23-25]. Therefore, it is unlikely that antibodies used as therapeutics for the targeting of immunomodulatory molecules, can reach all parts of the tumor in the brain and exert any effect unless they interact with target molecules in the periphery [26]. Of note, attempts to circumvent or block BBB drug-efflux activity to improve standard and targeted GBM treatments are currently being investigated [27].

Since it has long been believed that a classical lymphatic drainage system is absent in the CNS, it has been assumed that brain immune-surveillance occurs mainly in the meningeal compartment. Yet, recent evidence has questioned this long-held belief and shed new light on immune cell trafficking between the brain and extracranial sites. Indeed, two laboratories have independently found a dural lymphatic vascular system that drains fluids, macromolecules and immune cells from the cerebrospinal fluid, and is connected to the deep cervical lymph-nodes (dcLN) in rodent models [28, 29]. Although further experiments are required to finally confirm the functional trafficking of T cells to and from the human brain, the existence of a cellular route is further supported by single reports suggesting dcLN metastasis in primary brain tumors [30]. The discovery of the dural lymphatic route, along with the recently

described glymphatic system - a cellular pathway which facilitates CSF drainage and clearance of potentially toxic metabolites and unfolded proteins such as β-amyloid and tau protein into the brain parenchyma – suggests that the brain cannot be seen as an immune-privileged organ anymore but rather an immune-distinct site which is still accessible for immunotherapeutic approaches [31].

3. Mechanisms of immunosuppression in glioblastoma

3.1 Immunosuppressive immune cell subsets

3.1.1 Regulatory T cells

Regulatory T cells (Treg) account for 5-10% of all circulating $CD4^+$ T cells and are key modulators of the immune system maintaining tolerance to self and host antigens and inhibiting autoimmunity through resolution of tissue inflammation [32]. This T cell subset is characterized by the constitutive expression of the nuclear transcription factor forkhead box P3 (FoxP3), interleukin-2 receptor alpha chain (CD25), cytotoxic T lymphocyte antigen 4 (CTLA-4) and the glucocorticoid-induced tumor necrosis factor (TNF) receptor (GITR) [33]. Two main types of Treg exist: natural Treg (nTreg) which have developed in the thymus and induced Treg (iTreg) arising from FoxP3 induction in conventional $CD4^+$ T cells exposed to an immunosuppressive microenvironment. Despite marked lymphopenia, the Treg fraction in the peripheral blood and tumor specimens of glioma patients is increased and correlates with tumor grade and poor prognosis [34-36]. However, Treg presence in the blood or tumor as negative prognostic factor remains controversial since low percentage and no impact

on survival was observed in several other studies [20, 37, 38]. Discrepancies may be due to the existence of different Treg subtypes that can only be dissected by high-dimensional analyses and not only based on FoxP3 staining. In experimental models, glioma-infiltrating Treg are mostly thymus-derived nTreg as tumor entrance is drastically impaired in previously thymectomized mice [39]. This finding supports the idea that the glioma microenvironment efficiently recruits nTreg from the periphery, mainly through the chemokine/chemoreceptor axis C-C motif chemokine ligand 22 (CCL22)-C-C motif chemokine receptor 4 (CCR4), rather than switching conventional cells into iTreg [40]. In a transgenic mouse model of spontaneous astrocytoma it was observed that in early stage mice before symptom onset, $CD4^+$ and $CD8^+$ T cells were primarily recruited to the tumor with a significant proportion being $CD4^+$ $CD25^+$ T cells which comprise Treg [41]. To date, efficient targeting of the Treg population has been achieved with monoclonal antibodies (mAb) directed against CD25. Treatment of SMA-560 glioma-bearing VM/Dk mice with anti-CD25 mAb resulted in functional inactivation of Treg and increased anti-glioma immunity [34]. Furthermore, a humanized antibody against CD25 showed a good safety profile in patients with GBM and warrants further investigation in clinical trials [42]. A reduction of Treg infiltration in the tumor microenvironment may also be achieved indirectly by other therapeutic strategies. A vaccine generated by dendritic cells (DC) pulsed with tumor cell lysate induced a shift in the brain tumor immune cell infiltrate from Treg to T effector cells [43]. Of note, only vaccines derived from GL-261 murine glioma cells pre-treated with a combination of hypericin and light irradiation induced changes in the infiltrating T cell repertoire. This may be due to the fact that this treatment is supposed to elicit immunogenic cell death, which ultimately fosters anti-tumor responses [44]. Similarly, inhibition of transforming growth factor (TGF)-β signalling decreased the level of brain tumor-infiltrating Treg most likely as a consequence of

reduced trafficking from the periphery [45]. Data published by Lowther and colleagues suggest that a dysfunctional Treg population, marked by high expression of programmed cell death 1 (PD-1), has impaired suppressive activity and secretes high levels of IFN-γ [46]. This finding follows the discovery that Treg cells may drive tissue repair through the secretion of amphiregulin in response to inflammatory signals [47, 48]. Altogether, these observations reflect a broader role played by these cells in tissue inflammation and homeostasis, and challenge the view of a sole direct suppressive effect on antitumoral responses.

3.1.2 Tumor associated microglia/macrophages and myeloid-derived suppressor cells

Microglia and macrophage cells are the dominant immune infiltrate in gliomas and have been extensively studied for their key role in tumor pathogenesis [49, 50]. They can constitute up to 12% of the tumor mass and accumulation of these cells correlates with glioma tumor grade [51, 52]. These cells are part of the tumor mass and re-wired to secrete pro-tumorigenic factors.

Classically, microglial cells and macrophages have been distinguished by the cell surface marker CD45 with microglia being $CD45^{low}$ and macrophages $CD45^{high}$. In GBM, the $CD45^{high}$ population represents the largest fraction, even though microglial cells can upregulate CD45 levels during inflammatory conditions precluding a definite differentiation [53, 54]. To date, there are no generally accepted markers to distinguish these two cell populations that are characterized by a broad phenotypic plasticity. In general, two main phenotypes of macrophages and microglia exist: classically activated M1 cells, which have a marked effector activity against tumor cells and produce high amounts of pro-inflammatory cytokines, and alternatively

activated M2 cells, which tune down immune activation promoting tissue remodelling and repair. Notably, M1 and M2 cells represent only the extreme opposite of a continuous phenotypic spectrum [55]. Remarkably, this paradigm has been challenged by recent studies and the existence of these two polarized phenotypes has to be carefully reassessed in the future [56]. In GBM, a high percentage of tumor-associated macrophages (TAM) displays an immunosuppressive M2 phenotype that is characterized by the secretion of various molecules such as TGF-β, VEGF, epithelial growth factor (EGF), matrix metalloproteinase (MMP)-2 and MMP-9, which can promote tumor cell immune evasion, invasion, proliferation and angiogenesis (see below for details) [57-62]. Colony stimulating factor-1 receptor (CSF-1R) is expressed on several myeloid compartment cells. Binding of CSF-1 ligand promotes macrophage differentiation and survival, and is associated with sustained pro-tumorigenic functions mediated by this cell population. Targeting CSF-1R increased the survival of glioma-bearing mice through re-polarization of macrophages to a pro-inflammatory M1 phenotype [63].

Myeloid-derived suppressor cells (MDSC) represent another cell population with immunosuppressive properties. This immature myeloid subpopulation is recruited from the bone marrow and exerts inhibiting effects on cytotoxic T cells through nitric oxide (NO) production as demonstrated in a rat glioma model. Their presence is associated with an overall reduced number of tumor-infiltrating lymphocytes in murine and human gliomas [64, 65]. MDSC recruitment and function in the tumor is mainly driven by hypoxia, a key characteristic of glioblastomas [66]. In this regard, Du and colleagues demonstrated that hypoxia-driven hypoxia inducible factor (HIF)-1α expression induces the influx of MDSC to the tumor site which, in turn, enhances tumor angiogenesis and invasion [67]. Noman *et al* showed that HIF-1α has a binding site in the promoter of the programmed cell death ligand (PD-L)1 gene [68]. Hence,

HIF-1α upregulation may directly induce PD-L1 protein expression in tumor-infiltrating MDSC exposed to hypoxic conditions linking the suppressive function of these cells to the PD-1 pathway (see below). Besides, the immunosuppressive activity of both TAM and MDSC is directly fostered by a population of tumor cells with stem cell characteristic, also known as glioma-initiating cells, through the release of soluble growth and differentiation factor (GDF)-15, TGF-β1 and macrophage migration inhibitory factor (MIF) [69, 70]. In summary, myeloid compartment-derived cells which are present in glioblastomas have overlapping functions and intrinsic phenotypic plasticity. Furthermore, technical limitations regarding cell fate mapping as well as the lack of a commonly accepted nomenclature represent important issues that need to be addressed for an improved understanding of the functional role of these cells in the immunology of glioblastoma.

3.2 Glioma cell-derived secreted immunosuppressive factors

Glioma cells contribute to the immunosuppressive microenvironment through the secretion of various soluble factors (Figure 1). Discovered as *glioblastoma derived T cell suppressor factor* (GsT) because of its ability to impair interleukin (IL)-2-mediated T cell survival, TGF-β_2 was among the first identified immunosuppressive molecules released by glioma cells [71]. Three TGF-β ligand isoforms exist and are expressed by glioma cells [72]. To date, mainly TGF-β_1 and -β_2 have been described as modulators of immune cell function in the tumor micromilieu [73].

In the presence of TGF-β, $CD4^+$ T cells up-regulate FoxP3 and differentiate into Treg cells. Furthermore, $CD8^+$ effector T cells are profoundly impaired by TGF-β through activation of a repressive SMAD/ATF1-mediated transcriptional program, which inhibits the expression of several genes such as *IFN-γ* and *granzyme B* which are

required for the generation of a powerful anti-tumor immune response [74-76]. Moreover, TGF-β can directly down-regulate the expression of NKG2D, one of the most prominent activating natural killer (NK) cell receptors also conferring a co-stimulatory signal to $CD8^+$ T cells. Additionally, TGF-β decreases the expression of the NKG2D ligands MICA and ULBP2 on the tumor cell surface, further protecting glioma cells from immune-mediated destruction [77, 78]. TGF-β can polarize myeloid compartment cells into a M2 pro-tumorigenic phenotype, and impair antigen presentation by DC [79, 80]. Importantly, other TGF-β super-family members, such as GDF-15, also exert immune evasive effects in experimental gliomas [81]. Because of its strong immunosuppressive properties as well as further effects contributing to the malignant phenotype of glioma cells such as increased migration and invasion, angiogenesis, proliferation and maintenance of the stem-cell phenotype, TGF-β has been regarded an attractive therapeutic target [82-86]. However, despite many promising results in pre-clinical models, translation of TGF-β inhibition into clinical practice has remained challenging and a phase II trial in patients with recurrent GBM using the serine/threonine kinase inhibitor galunisertib, which blocks the intracellular cascade signalling initiated by TGF-β ligands through TGFβRI, did not improve progression-free or overall survival [45, 87-90]. It remains to be determined whether other strategies aiming at inhibiting TGF-β activity translate into clinical benefit or whether TGF-β inhibition alone is insufficient to mount significant anti-tumor immune responses.

Beyond TGF-β, many other glioma cell-secreted factors have been discovered. It has been shown that VEGF it is not only involved in glioma angiogenesis but can also hamper the maturation and function of APC and induce the expression of inhibiting receptors, such as PD-1 and CTLA-4, on $CD8^+$ T cells, negatively regulating their effector activity in the tumor microenvironment [91, 92]. Other factors and cytokines

with immunosuppressive functions released by glioma cells are IL-10, regeneration and tolerance factor (RTF), CSF-1, NO, prostaglandin E-2 (PGE-2) and arginase I. IL-10 may induce the expression of PD-L1 on monocytes while RTF may protect glioma cells from NK cell-mediated killing as shown in preclinical models [93, 94]. Levels of arginase I in the serum have been associated with immunosuppression in GBM patients whereas adult neural stem/progenitor cells-derived NO can suppress T cell function along with PGE-2 [95, 96]. Notably, the expression of some of these glioma-secreted factors may be upregulated following standard therapy treatment [97] which must be considered when designing future trials that combine conventional therapies with novel immunotherapeutic approaches [98].

3.3 Glioma cell membrane-bound factors with immunosuppressive function

Glioma cells can inhibit immune responses by direct cell-cell interactions involving several cell membrane-bound factors. One of the best characterized inhibitory pathways is represented by the axis formed by PD-L1, present on the tumor cell surface, and its cognate receptor PD-1 found on activated T cells. PD-1 plays a key role as immunological “rheostat”, dampening immune cell reactivity and maintaining immunological tolerance [99]. When bound to its ligands, PD-L1 or PD-L2, the SH2-domain containing tyrosine phosphatase (SHP)-2 protein is recruited in proximity of the antigen receptor signalling complex where it impedes the downstream activation pathway and induces exhaustion [100]. Thus, PD-L1-expressing tumors can generate a barrier against cytotoxic T cells in a protective process termed “molecular shield” [101]. Paramount importance of this axis has been demonstrated by durable and unprecedented clinical responses achieved by blocking PD-1 signalling with monoclonal antibodies in tumors such as metastatic melanoma and non-small cell

lung cancer [7, 102]. Although the activity of anti-PD1 antibodies against tumors residing in the brain is yet to be determined, these promising results and long-lasting responses prompted clinical evaluation of PD-1 inhibitors also in the context of newly diagnosed and recurrent glioblastoma [103, 104].

At the molecular level, the two ligands have a different expression pattern. PD-L1 is ubiquitously expressed and studies on its promoter revealed that two IFN regulatory factor (IRF)-1 binding sites exist. In GBM, the common phosphatase and tensin homolog (PTEN) loss has been linked with increased post-transcriptional expression of PD-L1. Conversely, PD-L2 expression is restricted to few activated immune cell populations and some tumor cell types but not glioblastoma [99, 105]. Several studies have proven the expression of PD-L1 in glioma cells *in vitro* and *in vivo* [106-110]. PD-L1 levels are dramatically increased in the presence of IFN-γ, one of the master immune effector cytokines. This process is called adaptive resistance and its physiological function is to secure the integrity of normal tissue during inflammation. Tumors hijack this system to escape from immune recognition and attack [111]. Striking survival benefits and even complete tumor eradication have been achieved in pre-clinical glioma models using PD-1 blockade either alone or in combination with radiotherapy or other immune checkpoint inhibitors [112-114]. Although these results highlight the potential importance of this pathway in the immunobiology of gliomas, the value of PD-L1 expression as a clinical prognostic factor as well as its use as biomarker for response to treatment with a PD-1 inhibitor remain controversial. Importantly, this ligand is not only present on tumor cells but can be virtually expressed by all cell types residing in the tumor microenvironment. For example, an immunosuppressive state can be induced by TAM through PD-L1 action [93]. Furthermore, an IFN-β-mediated autocrine loop increases the expression of PD-L1 in neurons and has been linked with glioma cell death induction and prolonged survival

of glioma patients [115]. PD-L1 may also confer reverse signalling into the tumor cell promoting resistance to apoptotic stimuli and may thereby be involved in the regulation of glucose metabolism [101, 116]. Hence, this ligand may represent a crucial cancer-promoting hub active during both tumor onset and subsequent immune escape and persistence.

Another molecule present on the cell surface of glioma cells is CD95 (Fas) ligand. This molecule can protect tumor cells from immune cell attack by inducing apoptosis in tumor-infiltrating lymphocytes following binding to its receptor on T cells [117-119]. Promising data in pre-clinical models prompted further investigation in human patients. APG101, a CD95 ligand binding fusion protein, has been evaluated in a randomized phase II trial in GBM patients with first or second tumor recurrence yielding promising results [120].

Glioma cells can also express non-classical major histocompatibility complex (MHC) class I molecules and directly inhibit immune cells. In GBM, HLA-E and HLA-G are mediators of glioma-induced immune cell inhibition. HLA-E is expressed by long-term and glioma-initiating tumor cells and inhibits NKG2D-mediated immune cell killing [121, 122]. Similarly, HLA-G expression interferes with efficient T cell priming and protects glioma cells from alloreactive and antigen-specific killing. Intriguingly, few HLA-G-positive cells within a HLA-G negative population were enough to exert significant immunosuppression *in vitro* [123]. Another molecule that represses NK cell activity against glioma cells is lectin-like transcript-1 (LLT-1), a ligand for the inhibitory NK cell receptor CD161. LLT-1 is expressed by glioma cells and is further induced by TGF-β. Silencing of this ligand restored *in vitro* NK cell-mediated killing of glioma cells [124].

3.4 Tumor cell metabolism

In normal cells, glucose is metabolized into pyruvate, which then enters the mitochondria as substrate to generate ATP through the process of oxidative phosphorylation. Conversely, most cancer cells produce energy by fermenting glucose into lactate, which exerts pleiotropic effects on the tumor microenvironment. It inhibits anti-tumor immune cell reactivity by several means including the inhibition of NK cell activity *via* down-regulation of perforin and granzyme B expression as well as an increased recruitment of bone marrow-derived MDSC. Furthermore, it impairs T cell activation and killing [125, 126] . Similarly, lactate dehydrogenase, which catalyses the reversible conversion of pyruvate into lactate, is highly expressed in hypoxic tumor regions thereby fostering immune evasion through NKG2D ligand induction in tumor-infiltrating myeloid cells and circulating monocytes [127].
Probably the most important metabolic pathway in glioma cells contributing to the immunosuppressive microenvironment is tryptophan catabolism. Tryptophan is an essential amino acid that can be metabolized into kynurenine by indoleamine-2,3-dioxygenase (IDO). Glioma cells express IDO which depletes tryptophan. Both tryptophan shortage as well as the production of kynurenine and similar catabolites can induce T cell anergy and immune cell apoptosis [128, 129]. IDO expression is associated with poor prognosis in GBM and expression of IDO in glioma cells resulted in increased Treg recruitment to the tumor in a preclinical animal model. In contrast, IDO-deficient tumor cells were efficiently eradicated by the immune system [130]. IDO has therefore been regarded as a promising therapeutic target. However, a challenge linked to IDO targeting is the presence of two other enzymes which may compensate its function: IDO2 and tryptophan-2,3-dioxygenase (TDO). Particularly, kynurenine produced by TDO may contribute to tumor cell survival and suppresses anti-tumor immune responses through the activation of the aryl hydrocarbon receptor

(AHR) signalling pathway. The TDO-AHR axis was found to be active also in human brain tumors such as glioblastoma and linked to poor survival [131].

4. Conclusions and outlook

Glioblastoma is paradigmatic for its ability to interfere with anti-tumor immune reactivity. In a comprehensive analysis using large-scale genomic datasets of different tumor entities, Rooney *et al* demonstrated that GBM has the lowest "*immune cytolytic activity*" amongst all neoplasias [132]. Yet, the immune cytolytic activity in GBM is higher than in low-grade gliomas and normal brain tissue, suggesting that there is an interaction between the immune system and the tumor cells.
GBM imposes a tight local immunosuppressive network by virtue of an intertwined assembly of immunosuppressive mechanisms (Figure 2). T cell priming, activation and killing, is restrained in a paracrine-fashion through secretion of TGF-β and other soluble molecules, and by direct cell-cell contacts involving PD-L1, CD95-L or non-classical MHC class-I proteins. Treg and MDSC cells are recruited to dampen anti-tumor immune activity and macrophages as well as neutrophils are skewed toward a tumor-promoting phenotype. The tumor mass is associated with a detrimental hypoxic environment which may further support malignant clonal selection and immune impairment. Aberrant metabolic pathways withdraw key nutrients such as glucose and tryptophan, and result in immunosuppressive metabolites leading to T cell anergy and apoptosis. Looking at this complex situation, it becomes clear that exploiting the immune system for therapeutic purposes may require therapeutic combinations aiming at blocking major GBM-derived inhibitory hubs as well as a re-invigoration of innate and adaptive immune reactions by additional measures such as vaccination. Possibly, it will even be required to apply *ex vivo* manipulation of

immune cells and subsequent re-infusion to patients. Some of these concepts are currently being explored in a therapeutic approach involving chimeric antigen receptor (CAR) cell technology. Here, naïve T cells are transduced with a construct expressing a receptor for a specific antigen. T cells bearing a CAR can be activated by this antigen in the absence of any co-stimulation. The recognized antigen should ideally be expressed exclusively by tumor cells to avoid any collateral damage to normal tissues. Currently, phase I/II clinical trials are exploiting CAR T cells targeting the glioma-specific antigens EGFRvIII and IL13Rα2 (NCT02209376; NCT01082926), which have demonstrated promising results in preclinical models [133, 134]. Further clinical strategies and challenges associated with these immunotherapeutic approaches have been reviewed elsewhere [104, 135, 136]. From the therapeutic perspective, improvement of drug delivery across the BBB may also be crucial to increase the efficacy of immunological treatments. Finally, a deeper understanding of the immunological routes from the periphery to the brain may allow for the design of new therapeutic targets and strategies.

So far, immunotherapy for brain tumors has been largely disappointing. However, in light of the increased knowledge on tumor-derived immunosuppressive pathways as well as the availability of novel drugs such as vaccines and checkpoint inhibitors, a reappraisal of the available and potentially useful immunotherapeutic approaches is warranted (Figure 3). Well-designed clinical trials accompanied by appropriate translational research efforts aiming at understanding which patients may benefit from immunotherapeutic treatment may result in clinical progress in the near future.

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Figure Legends

Figure 1. **Glioma cell-derived secreted factors.** Glioma cells can release several soluble mediators which exert a variety of immunosuppressive effects. Transforming growth factor (TGF)-β inhibits transcription of IFN-γ, granzyme B (GZMB) and NKG2D in immune effector cells, induces the alternative activation of macrophages (M2) and mediates regulatory T cell (T_{REG}) induction and recruitment from the periphery. Vascular endothelial growth factor (VEGF) impairs the antigen-presentation capacity of dendritic cells and increases the expression of immune inhibitory checkpoint molecules on the surface of T effector cells, such as programmed cell death (PD)-1. Growth and differentiation factor (GDF)-15, IL-10, regeneration and tolerance factor (RTF), colony stimulating factor (CSF)-1, nitric oxide (NO), prostaglandin E-2 (PGE-2) and arginase I (ArgI) generate an immunosuppressive environment which hampers anti-tumor immune activity.

Figure 2. **Cellular and molecular pathways of immunosuppression.** Regulatory T cells (T_{REG}) and alternatively activated macrophages (M2) and neutrophils (N2) interfere with the anti-tumor activity of natural killer (NK) and cytotoxic T cells (**A**). IFN-γ secreted by T cells to kill tumor cells can induce the expression of programmed cell death ligand (PD-L)1. This ligand binds its cognate receptor programmed cell death (PD)-1 expressed on activated T cells ultimately leading to T cell anergy and apoptosis. PD-L1 can also be expressed on myeloid-derived suppressor cells (MDSC) that are recruited to hypoxic regions of the tumor (**B**). The essential amino acid tryptophan (L-Trp) is crucial for proper T cell activation and effector function. Glioma cells express the enzymes indoleamine-2,3-dioxygenase (IDO) and tryptophan-2,3-dioxygenase (TDO) which can consume this amino acid restricting T cell metabolism. Kynurenine (Kyn), a catabolic product of L-Trp metabolism, is toxic for T cells and binds to the aryl hydrocarbon receptor (Ahr) mediating tumor cell

survival (**C**). Non-classical MHC class-I molecules such as HLA-E and HLA-G as well as lectin-like transcript-1 (LLT-1) are expressed on the tumor cell surface and mediate glioma cell immune-evasion (**D**).

Figure 3. **Therapeutic targeting of the immunosuppressive microenvironment.** Alternatively activated macrophages (M2) activity could be reversed by colony stimulating factor 1 receptor (CSF-1R) inhibitors, whereas regulatory T cells (T_{REG}) are depleted by treatment with antibodies targeting CD25. VEGF effects on tumor vasculature and immune cells may be blocked by the monoclonal antibody bevacizumab. Small molecules targeting the TGF-β receptor 1 (TGFβRI) can relieve the immunosuppressive effects exerted by TGF-β on T cells and prevent its tumor cell-intrinsic pro-tumorigenic functions. Antibodies targeting PD-1 or its ligand PD-L1 may reinvigorate exhausted T cells restoring their activity against tumor cells. 1-methyl-D-tryptophan (1-D-MT) is an indoleamine-2,3-dioxygenase (IDO) inhibitor which may exert anti-tumor effects in combination with a chemotherapeutic agent.